Supplementary Methods

Transgenic mice. Gin transgenic mice\textsuperscript{10} were obtained from the Jackson Laboratory. In G42 mice, a GAD67 BAC construct directs GFP expression in a subset of parvalbumin positive basket interneurons in visual cortex (Chattopadyhyaya et al., in press). We have no indication that chandelier cell are labeled in G42 mice.

Acute brain slices. Coronal slices (350 µm) of visual cortex were prepared from postnatal day (P)28-P34 G42 and Gin transgenic mice. Briefly, after sectioning in ice-cold oxygenated (95% O2/5% CO2) dissection buffer (in mM: 212.7 sucrose, 5 KCl, 1.25 NaH2PO4, 3 MgCl2, 1 CaCl2, 26 NaHCO3 and 10 dextrose), slices were transferred to a storage chamber containing ACSF (in mM: 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1 MgSO4, 2 CaCl2, 10 D(+)-glucose, 25 NaCHO3; continuously bubbled with 95%O2-5%CO2) for at least 1 hr before imaging. During the imaging session, slices were perfused with oxygenated ASCF at a rate of 2-3 ml/min (33±1 °C).

Organotypic cortical cultures. Postnatal day 2–5 mouse pups were decapitated, and the brains rapidly removed and immersed in ice-cold artificial low sodium cerebrospinal fluid (ACSF, containing 4 mM KCl, 5 mM MgCl2, 1 mM CaCl2, 26 mM NaHCO3, 10 mM glucose and 8% sucrose, saturated with 95%:5% O2:CO2). Coronal brain slices of parietal and occipital cortex, 400 µm thick, were cut with a Chopper (Stoelting) into ice-cold ACSF. Slices were then placed on transparent Millicell membrane inserts (Millipore, Bedford, MA), usually two slices/insert, in 30 mm Petri dishes containing 1 ml of culture medium (containing DMEM, 20% Horse serum, 1 mM Glutamine, 13 mM Glucose, 1 mM CaCl2, 2 mM MgSO4, 0.5 µm/ml Insulin, 30 mM Hepes, 5 mM NaHCO3 and 0.001% Ascorbic acid). Finally, they were incubated in a humidified incubator at 34°C with a 5% CO2 enriched atmosphere, and the medium changed thrice a week. All procedures were performed under sterile conditions. Slices were imaged at Equivalent Postnatal Day (EP) 28-32.

Single cell electroporation. Glass micropipettes (with filament) with tip size around 0.6-1 µm and resistance about 10 MΩ were used. The pipette was filled with 5 mg/ml Dextran-Texas Red 3000MW (Molecular Probes) in standard ACSF. Infrared DIC optics was used to locate pyramidal neurons postsynaptic to GFP labeled axon. Grass
SD9 stimulator was used to deliver exponential decay pulses with peak voltages of 8 V, duration 1 msec, frequency 200 Hz, 4 msec delay. Two trains of such pulses with 1 second in duration were delivered for each electroporated cell. Only well-filled pyramidal cells were selected for analysis. To define pyramidal neurons we used the following criteria: presence of an apical dendrite, presence of numerous and prominent spines, initial trajectory of the axon towards the white matter. We aimed to electroporate single pyramidal cell in layer 5/6 because GFP expressing basket cells in G42 mice are mainly localized in layer5/6 (not all the parvalbumin positive cells express GFP). For consistency, we also studied layer5/6 pyramidal cells in GIN mice. The filling of distal branches of long apical dendrites of pyramidal neurons were in some cases incomplete, we thus focused our study only on basal dendrites which were well and completely filled.

Two-photon imaging: Living slice preparations were imaged using a custom-built 2-photon laser scanning microscope based on a Fluoview laser scanning microscope (Olympus America Inc., Melville NY). We used a 60X objective (NA 0.9, Olympus) and the light source was a Ti:Sapphire laser (Mira, Coherent) running at a wavelength of 910 nm. Fluorescence was detected in whole field detection mode with a photomultiplier tube (Hamamatsu, Bridgewater NJ). Laser power was adjusted so that additional power failed to reveal previously undetected boutons and spines. Optical sections were collected at 0.8 μm spacing.

Data analysis: Putative synaptic contacts between GFP-labeled interneuron boutons and pyramidal cell dendrites were reconstructed from image stacks using the confocal module of Neurolucida (Microbrightfield). Each putative contact was evaluated for overlap between green and red signal in three dimensions. Supplementary Fig.2b shows a bouton contacting the soma of a pyramidal cell in both the xy and yz planes. For contacts along dendrites (Supplementary Fig.2c), the red and green fluorescence intensity was plotted along a line across the juxtaposition of the dendrite and a bouton in the focal plane in which the green signal was higher (Supplementary Fig.2c1). A contact was judged as such when the overlap between the green and red curves (from which background level are subtracted) was at least 500 nm (Supplementary Fig.2c2). This value was chosen by considering the size of synaptic cleft and the optical
resolution of our imaging system. This analysis was done blind to genotypes (G42 vs Gin) and conditions (acute vs cultured slices).

To determine whether anatomically identified GFP-positive boutons correspond to synaptic site, cortical slices from both transgenic lines were fixed and immunostained with antibody against GAD65 (mouse anti GAD65, 1:1000; Chemicon), a well established GABAergic presynaptic marker. Quantitative analysis of confocal images showed that 82% (82 out of 100 analyzed boutons) of the GFP positive boutons in G42 mice and 82% (87 out of 106 boutons) in GIN mice coexpressed GAD65 (Supplementary Fig.3a,b). To further investigate this point, cortical slices were immunostained with a GFP antibody followed by DAB reaction. Putative boutons were identified under light microscopy and then analyzed using immuno-electron microscopy (Chattopadhyaya et al, in press). All GFP positive boutons analyzed contained synaptic vesicles and formed symmetric synaptic contacts. For example, Supplementary Fig.3c shows a GFP-expressing bouton in GIN mice forming synaptic contact onto a spine. Therefore, most GFP positive boutons likely correspond to putative presynaptic sites. It remains possible that these “putative synaptic contacts” are a slight over estimate of synapses. However, such overestimate should apply to all cellular compartments in both acute and slice cultures, and therefore should not affect the relative distribution of synapses among different cellular compartments.

The dendritic arborization of each pyramidal cell was divided in 6 “compartments”: soma, 0-40 µm from soma, 40-80 µm from soma, and so on. For each pyramidal cell, the number of contacts in each compartment was normalized to the total number of contacts found for that cell. This procedure allows us to determine the distribution of contacts along the dendrites independently from individual differences in the number of GFP-expressing interneurons. Results for pyramidal cells reconstructed from the same transgenic line were not significantly different and were pooled. To normalize bouton distribution against dendritic length in each cellular compartment (i), we calculated an index which took into account the dendritic length in that compartment, and plotted the mean index for each experimental group against each dendritic segment. Dendritic Index (DI) was calculated as following: DI\(i\) = \(\{(N_i \text{ contacts}/N_{tot} \text{ contacts})/(\text{dendritic length in }\)
compartments i/total dendritic length). Differences in the distribution of synaptic contacts between 2 groups were assessed with Chi-square test. Differences between all four experimental groups were assessed with Kruskal-Wallis one-way ANOVA with Tukey's *post hoc* test for normally distributed data and with Kruskal-Wallis one-way ANOVA on ranks with Dunn's *post hoc* test for not normally distributed data.